STUDIES ON THE PHYSIOLOGY OF UCA RED CHROMATOPHORES [1]

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Red chromatophores are present in considerable abundance in most individuals of *Uca pugilator* that are collected in the salt marshes in and near Woods Hole, Massachusetts. The abundance is sufficient to provide the animals with a distinctly reddish tint. Almost nothing has been reported concerning the responses of these chromatophores to background or to other factors, nor have any systematic studies been made as to the nature of the control of these chromatophores within the organism. Carlson (1936) reported that the red pigment was concentrated in eyestalkless *Uca pugilator*. This was confirmed by Abramowitz (1937) who reported further that injection of eyestalk extract into eyestalkless specimens caused red dispersion.

In view of the rapidly increasing information on the physiology of the black and white chromatophores of the fiddler crabs, and the various sources of chromatophorotropins involved, it seemed wise at this time to examine the red chromatophores, comparing them with the other chromatophores in their responses, to learn to what extent they were governed by the same or similar mechanisms, and to what extent by quite different ones.

Responses to Black and White Backgrounds

A number of experiments were performed to determine the character and magnitude of changes in the state of red chromatophores in response to black and white backgrounds. In a typical experiment, twenty fiddler crabs were divided into two lots of ten each; one lot was placed in a black enamelled container, the second in a white one. After about three hours in these situations the average state of dispersion of the pigment of the red chromatophores was recorded, using the well-known chromatophore index in which a chromatophore with its pigment in punctate condition is designated as 1, and one with its pigment completely dispersed, as 5, with 2, 3, and 4 describing three intermediate states. The backgrounds of the two lots of animals were then interchanged and the average state of the red pigment in the two lots determined after one, two, and three hours. The results of this experiment, conducted at an illumination of 100 f.c., are shown in Figure 1. It is readily seen that the pigment responds extensively, in fact through almost its entire normal range of change, during the daytime, at constant incident illumination, and in response simply to these changes in albedo.

In the foregoing characteristic, the red chromatophores of *Uca pugilator* differ quantitatively in their responses from the black and white chromatophores, whose responses to albedo, when incident illumination, temperature, and time in the daily

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rhythmical cycle of color change are constant, are of relatively small magnitude (Brown and Sandeen, 1948).

The red chromatophores of *Uca,* therefore, appear in their responses well adapted for alteration of the shade of the body to accord more closely with that of the background upon which the animal is located.

Diurnal Rhythm of Red Chromatophore State

In conditions of constant darkness, the red chromatophores of *Uca pugilator* exhibit a very striking persistent rhythmicity of activity. This rhythm is illustrated in Figure 2A (solid line) in which it is readily observed that the pigment is more or less broadly dispersed by day and almost completely concentrated by night. The darkened blocks immediately above and below the plotted rhythm indicate the hours 6 P.M. to 6 A.M. The bottom band in the figure indicates the experimental illumination which, in this instance, is constant darkness. The amplitude of the rhythm was, in fact, substantially greater in this case than that of the rhythm simultaneously occurring in the black chromatophores of these animals (broken line in Figure 2A). The two rhythms are, however, clearly seen to be in phase with one another.

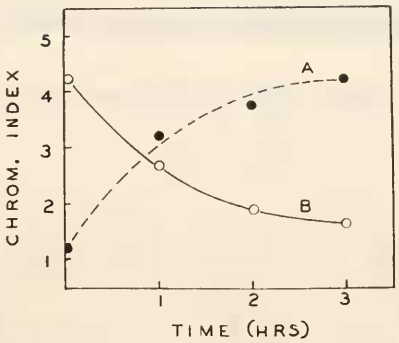

Figure 1. Changes in the red chromatophores in response to change of background: A. White to black; B. Black to white.

The rhythm of the red chromatophores was observed to persist with no loss in amplitude, and completely synchronized with the solar day-night cycle for four days.

In an attempt to demonstrate whether the red and black chromatophores were able, in a normal animal, to show activities in opposite directions to one another at any given moment, ten animals were exposed to twelve hours of illumination by night and to twelve hours of darkness by day over a period of three days. They were maintained during the experiment upon a white enamelled background. The results of this experiment are shown in Figure 2B between points *a* and *b*. The red chromatophores (solid line) showed dispersion of their pigment during the darkened day-time period, and concentrated their pigment during the illuminated night-time period in response to the white background.

The black chromatophores (broken line), on the other hand, governed predominantly by the endogenous persistent rhythmicity, altered the phase of their

rhythm in response to the reversed condition of periods of illumination in such a manner as to show their maximally concentrated phase at mid-day rather than at mid-night (Brown and Webb, 1948, 1949), and hence the rhythms of activity of the two pigments are seen to be out of phase with one another as long as the reversed periods of illumination are continued.

It is also seen from Figure 2B, beginning at point *b*, that when the animals are returned to constant darkness after the three complete cycles of reversed illumina-

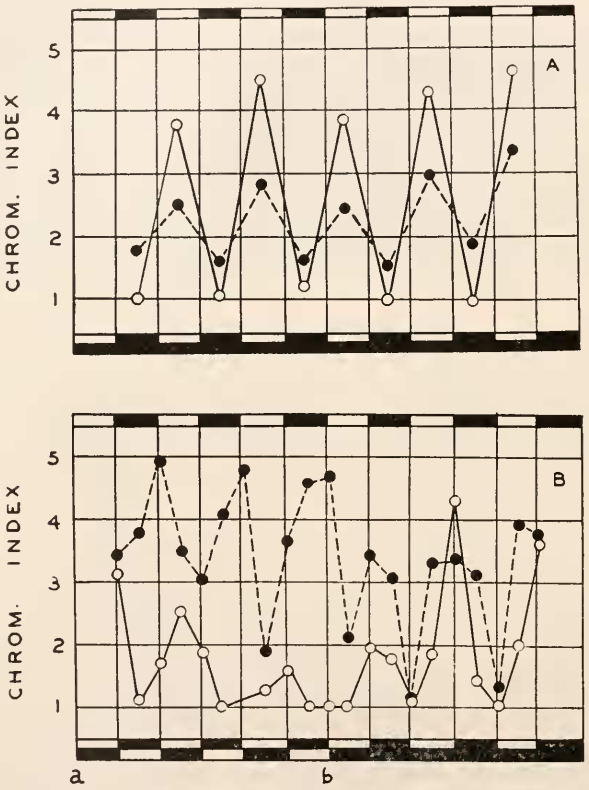

FIGURE 2. A. The normal persistent rhythmicity of red (solid line) and black (broken line) chromatophores in constant darkness. See text for explanation of diagrams.

B. Behavior of red (solid line) and black (broken line) chromatophores in response to a series of periods of reversed illumination beginning at *a*, followed by constant darkness beginning at *b*.

tion, the black pigment, as a result of a shift in the phase of the endogenous source of the rhythm, continues to exhibit a persistent 24-hour cycle of activity with maximum concentration of the pigment now at about 7 A.M. Under these conditions the red pigment also undergoes a similar rhythmical activity completely in phase now with the black. This rhythm was observed to persist unchanged for more than a week.

These results clearly indicate that the red pigment, other environmental stimuli removed, is under a very effective control by an endogenous mechanism of persistent

rhythmicity, and that in the absence of such other stimuli the various phases of red chromatophore activity are controlled and experimentally shifted in a manner quite parallel to that found for the black chromatophores.

It is also evident from these observations that the red pigment is capable of exhibiting an activity independent of that of the black; indicating, in addition, some difference in the factors contributing to their control.

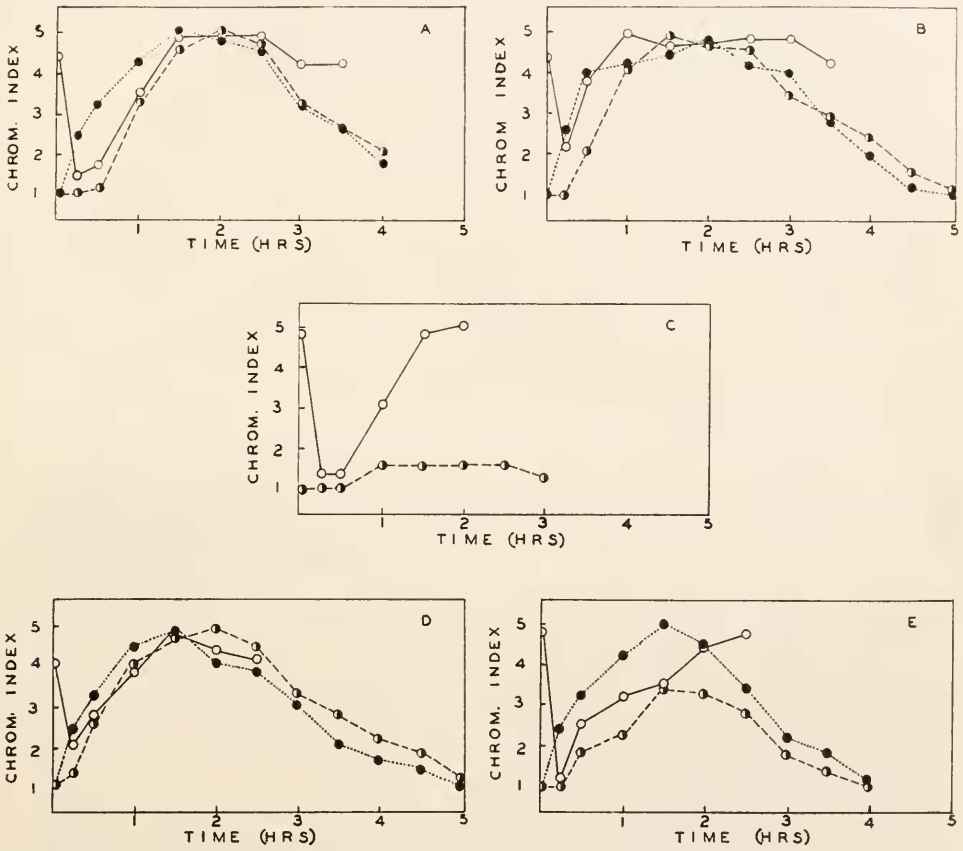

FIGURE 3. Responses of chromatophores of eyestalkless (red chromatophores—dashed line; black chromatophores—dotted line) and black-adapted intact *Uca* (red chromatophores—solid line) to injection of extracts of A. sinus glands, B. optic ganglia, C. circumoesophageal connectives, D. brain, and E. thoracic cord.

SOURCES OF RED-PIGMENT-INFLUENCING FACTORS

The sinus glands and various portions of the central nervous system appeared to be the most likely sources of chromatophorotropins influencing red pigment, in view of their demonstrated activities on white and black pigments in both this species (Brown, 1948) and another crab (Bowman, 1949). Consequently, a series of experiments was designed to determine what influences, if any, extracts of these organs might show.

Sinus glands, optic ganglia from which sinus glands had been carefully removed, brains, connectives, and thoracic cords were removed from a number of specimens and triturated in sea water in such volumes that a single dose (0.05 ml.) to be injected later into an assay animal would contain the equivalent of one-third of the complement of that tissue taken from a single animal. Such an extract was referred to as possessing a concentration of 1/3. The various extracts were then each assayed on (1) five *Uca pugilator* whose eyestalks had been removed 24 hours previously and whose red chromatophores were in an initial state of 1, and (2) five normal animals which were adapted to a black background and which were maintained upon a black background throughout the assay.

The states of the red chromatophores were determined at the time of the injection and then at 15 minutes, 30 minutes, and at 30-minute intervals thereafter until the chromatophores returned to their initial states.

The results of these experiments are summarized in Figure 3. The solid lines represent the averages for the black-adapted assay animals whose red pigment was initially dispersed, and the dashed lines represent the averages for the eyestalkless ones whose pigment was initially concentrated. The dotted lines show the behavior of the black chromatophores in the eyestalkless assay specimens.

It is readily seen that all of the extracts exhibited a substantial influence on the red chromatophores, an effect not shown either by sea-water injections or injections of extracts of muscle tissue which were used as controls.

The influence of all the active extracts showed two characteristics in common. The first was that they all exerted a rather strong concentrating influence upon the red pigment that was initially dispersed in the black-adapted assay animals. Undoubtedly the same type of activity accounted for the significant delay in the initiation of dispersion of red pigment in the eyestalkless assay animals whose red pigment was initially strongly concentrated. There is, in this latter connection, a rather close correlation between the magnitude of the red-pigment-concentrating action and the extent of this initial delay.

The second type of activity, one which was most conspicuously evident in the eyestalkless assay animals, was that of strong dispersion of red pigment. This action was also evident in the black-adapted animals in the form of the dispersion of the red pigment to a condition greater than that of the initial and final states, following the initial period of concentration.

Closer inspection of Figure 3 shows that the five extracts did not exhibit equal red-pigment-concentrating actions on the black-adapted assay animals. It can be seen that the apparent order of decreasing activity in this regard was as follows:

Thoracic cord > Connectives > Sinus glands > Brain > Optic ganglia

The foregoing order of red-concentrating activity was not the same, or even the simple inverse, of the order of red-pigment-dispersing activity which appears to be:

Optic ganglia > Brain > Sinus glands > Thoracic cord > Connectives

It would appear from these data that all of the portions of the nervous system, and the sinus glands as well, possess two different principles which are responsible for these two observed influences on red pigment.

Additional support for the dual activity of brain and thoracic cord extracts upon red pigment is seen in Figure 4, A and B, in which extracts of these organs were

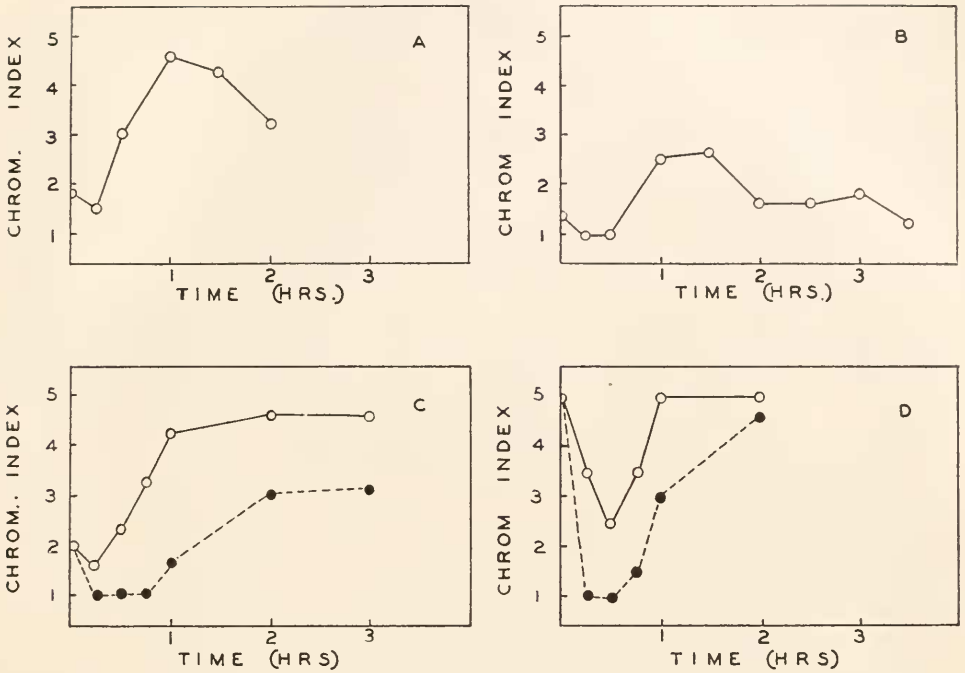

FIGURE 4. Responses of intact white-adapted *Uca* to extracts of A brain and B thoracic cord. C. Responses of the red chromatophores of eyestalkless *Uca* to alcohol-soluble (broken line) and alcohol-insoluble (solid line) fractions of sinus glands. D. Same as C but now responses of intact black-adapted *Uca*.

injected into normal white-adapted assay animals maintained upon a white background. Again, the same duality of activity of the extracts is observed.

A partial separation of these two principles appears to have been effected by the preparation of two types of extracts of sinus glands: one an ethyl-alcohol-soluble fraction and the other an ethyl-alcohol-insoluble fraction. Figure 4, C and D, shows the results of injecting these two fractions in equivalent concentrations into (C) eyestalkless assay animals selected to show slight red dispersal, and (D) black-adapted assay animals. In both instances the alcohol-soluble fraction appeared to possess a distinctly larger proportion of the red-pigment-concentrating activity, and the alcohol-insoluble fraction to possess the more black-dispersing action.

In an attempt to compare quantitatively the various portions of the nervous system and the sinus glands, one with another, with respect to their red-dispersing activity, extracts of sinus glands and of optic ganglia with sinus glands carefully removed were made up in concentrations of 1/3 as in the earlier experiments. A series of dilutions of these extracts was made to yield concentrations of 1/3, 1/9, 1/27, 1/81, 1/243, and 1/729. Each concentration of each organ was injected into five eyestalkless assay animals. This experiment was repeated three times. In Figures 5, A and B, are shown the relationships between the log concentration of the extract and the red-pigment-dispersing activity. The relationship is seen

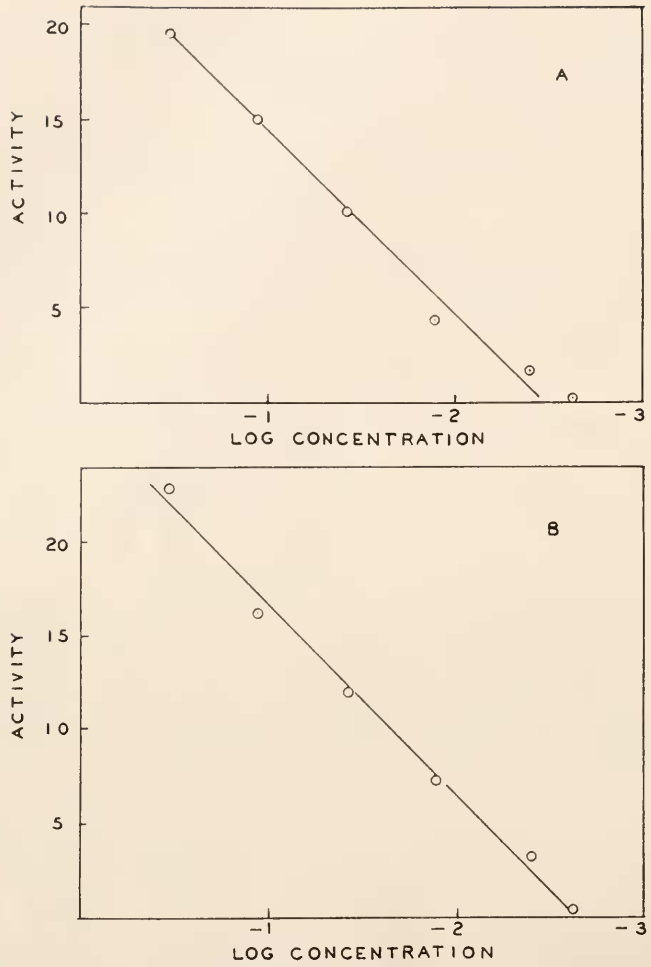

FIGURE 5. Relationship between extract activity on red chromatophores and log of concentration. A. Sinus gland extract. B. Extract of optic ganglia.

to be essentially a simple linear one. The activity was determined in the manner described by Brown and Sandeen (1948) in which the total of all the average chromatophore readings at 15 minutes, 30 minutes, and at 30-minute intervals thereafter until the end of the effect are added together. The difference between this sum and the corresponding sum at the same time intervals which would have indicated no activity was considered a reasonable measure of the activity since it incorporated both the amplitude and duration of effect of the injected extract. Using this dilution-series information to indicate the relationship between the concentration of the principle concerned and the amount of activity, and then using values of activities calculated in a similar manner from the data of Figure 3, it may be estimated that the optic ganglia and brain both contain approximately the same quantity of the principle. The sinus glands appear to possess only about one-half

as much as the brain. The thoracic cord contains about one-thirteenth the amount of the brain, and the connectives only about one-eighty-eighth of the amount in the brain.

It must be borne in mind that these values are still quite rough approximations in view of the calculations being based on the assumption that the amounts of the antagonist (red-concentrating substance) are in equal quantities in all portion of the nervous system, an assumption that is in all probability not justified.

Similarly, values for the relative quantities of red-concentrating principle within the various organs tested assume equal quantities of its antagonist (red-dispersing factor) in all portions.

More precise determination of the quantitative distribution of the two factors within the various tissues must obviously await the development of methods for the complete separation of these from one another.

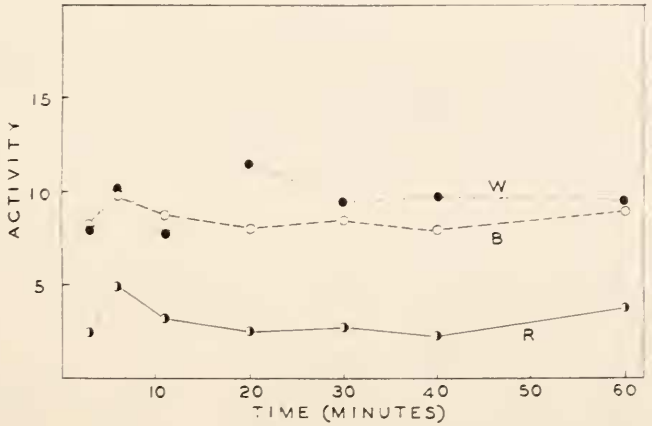

FIGURE 6. Alteration in activity of sinus-gland extracts on red (R), black (B), and white (W) chromatophores over the course of one hour following extraction.

ON THE PROBABLE IDENTITY OF THE BLACK- AND RED-DISPERSING PRINCIPLES

All the data which have been obtained in the course of these experiments upon the red chromatophores support an hypothesis that the red-dispersing principle from the sinus glands and the central nervous system is identical with the black-dispersing principle from the same organs. Two types of experiments have provided particular support.

In the first of these experiments, when eyestalkless assay animals were injected with extracts of various known sources of red-dispersing principle, the sinus glands, thoracic cords, brains, and optic ganglia, extracted in such proportions as to provide concentrations of 1/3, the effects upon the black chromatophores were observed simultaneously with those on the red. As can be seen from the results shown in Figure 3, A, B, D, and E, the action on the red chromatophores parallels in a remarkable fashion the action upon the black after the initial period of retardation. This initial retardation of dispersal has been seen earlier to be due to the simultaneous presence of a red-concentrating factor. Following the retardation the red

pigment usually overtakes the black, for which there has apparently been no similar concentrating principle present, and then the duration of the action of the extracts upon these two pigmentary types is virtually identical.

The second type of experiment involved a determination of the alteration with time of the activity of extracts of the connectives of *Uca* upon three chromatophore types. Extracts of connectives were made up to provide concentrations of 1/3. Five assay animals were injected after 3, 6, 11, 20, 30, 40, and 60 minutes and the activity of the extract determined at each of these times on the red, black, and white pigments. Figure 6 shows the average of the results of three such experiments. The parallel character of the activity of the extracts upon the red and the black chromatophores is conspicuous. In contrast with this, the relationship with respect to the white pigment appears to be different from those of the red and black, and other types of evidence have clearly indicated them to be two distinct substances.

Summary

1. The red chromatophores of *Uca pugilator,* when other factors are equal, exhibit extensive responses to background. They disperse their pigment upon a black background, and concentrate it upon a white one.

2. Under conditions of constant darkness the red chromatophores show a striking persistent daily rhythmicity, dispersing their pigment by day, and concentrating it by night.

3. Extracts of sinus glands, and all of the major portions of the central nervous system, possess strong activity upon the red chromatophores. All portions exhibit two types of activity due to the possession of two principles. One of the activities is red-concentration and the other, red-dispersion.

4. The action of the red-concentrating principle can dominate the response when there is, concurrently present, high concentrations of both red-dispersing and red-concentrating principles.

5. The red-concentrating activity of all extracts is of much shorter duration than is the red-dispersing activity, the latter often lasting 3 to 4 times as long as the former.

6. Evidence is presented which strongly suggests that the black-dispersing and red-dispersing actions are due to one and the same principle.

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